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Anaemia among primary care patients with Type 2 Diabetes Mellitus (T2DM) and chronic kidney disease (CKD): a multi-centred cross-sectional study

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ABSTRACT

Objectives:

This study aimed to determine the prevalence of anaemia among patients with T2DM and CKD at primary care settings and its associated factors.

Design, setting and participants:

This cross-sectional study involved 808 adult patients with T2DM and CKD who were recruited via systematic sampling from 20 public primary care clinics in Peninsular Malaysia. Their socio-demographic, clinical and biomedical profiles were collected through interviews, examination of medical records and blood testing.

Results:

The prevalence of anemia was 31.7% (256/808). The anaemia was mainly mild (61.5%) and normocytic normochromic (58.7%). About 88.7% of the anaemic patients were not known to have anaemia prior to the study. Among 36 patients with documented history of anaemia, 80.6% were still anaemic and only a half received iron therapy. Multivariate regression analysis showed that female (AOR: 1.56, 95% CI: 1.12-2.21, p=0.009) and those with older age (AOR: 1.04, 95% CI: 1.01-1.06, p<0.001), CKD Stage 3a (AOR: 2.47; 95% CI: 1.25-4.87, p=0.009), CKD Stage 3b (AOR: 4.36; 95% CI: 2.14-8.85, p<0.001), CKD Stage 4 (AOR: 10.12; 95% CI: 4.36-23.47, p<0.001), CKD Stage 5 (AOR: 10.80; 95% CI: 3.32-35.11, p<0.001) and foot complication (AOR 3.12, 95% CI: 1.51-6.46, p=0.002) were more likely to have anaemia. Having higher body mass index (adjusted OR 0.95, 95% CI: 0.92-0.99, p=0.012) and higher diastolic blood pressure (adjusted OR 0.97, 95% CI: 0.95-0.99, p<0.001) were associated with lower odds to have anaemia.

Conclusion:

Prevalence of anaemia among patients with T2DM and CKD in primary care was common and the majority was unrecognised. Inadequate treatment of anaemia was also prevalent. Therefore, screening of anaemia should be incorporated into the routine assessment of diabetic complications particularly for those with significant associated factors. It is hoped that such strategy could lead to early treatment and hence improve their overall care.

Keywords: Anaemia, diabetes mellitus, chronic kidney disease, primary care

Trial registration number: NMRR-15-660-24324

Strength and limitations of this study:

1. This was the first nationwide study to examine the prevalence of anaemia among patients with T2DM and CKD involving a large sample from multi-centres in Malaysian primary care setting.
2. The findings help to identify those who require simple anaemia screening using full blood count and this potentially cost-saving approach of screening may improve the care of such patients.
3. The findings could also provide a groundwork for future studies as previous studies on anaemia among patients with T2DM and CKD are still limited.
4. Unfortunately in this study, the actual aetiology of the anaemia was not determined thus the anaemia may be caused by causes other than renal anaemia.
5. Since this study did not include patients from the private primary care clinics and those in the east of Malaysia (Sabah and Sarawak), the prevalence of anaemia may be underestimated.

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Conflicts of interest: None to declare

INTRODUCTION

Chronic kidney disease (CKD) is common among patients with type 2 diabetes mellitus (T2DM). It is defined when there is an irreversible loss of kidney function for at least three months.[1] The estimated glomerular filtration rate (eGFR) and evidence of kidney damage have been used to diagnose CKD, which can be divided into five stages based on the National Kidney Foundation-Kidney Disease Outcomes Quality Initiative (NKF-KDOQI) staging.[1] The estimated prevalence of diabetic nephropathy worldwide ranges between 26% and 58%.[2-10] However, the prevalence appears lower in Malaysia as it ranges between 7.8% and 34.9%.[11-15] This wide range of prevalence may be due to difference in study settings and definition used for diabetic nephropathy. Nevertheless, data from the National Diabetes Registry Malaysia in 2012 involving 657,839 registered T2DM patients from government health facilities including hospitals and primary care clinics showed a prevalence of 7.8%.[14]

CKD has been associated with anaemia, cardiovascular disease, bone metabolism abnormalities, metabolic acidosis and malnourishments. Among these, renal anaemia has been increasingly studied over the past few years due to high all-cause mortality and cardiovascular morbidity associated with renal anaemia, as well as availability of erythropoietin stimulating agents (ESA) for treating renal anaemia.[16,17] In addition, anaemia has also been found to be a significant independent factor for progression of nephropathy to end stage renal failure among patients with T2DM.[18] Overall, renal anaemia carries significant burden to the sufferers as well as the healthcare system that bears the cost of care for patients with T2DM and CKD.

According to the Kidney Disease: Improving Global Outcome (KDIGO) Anemia Work Group, anaemia in CKD is when haemoglobin (Hb) level <13 g/dL for men and <12 g/dL for women.[19] It is caused by multiple pathophysiological mechanisms, which include erythropoietin deficiency, nutritional deficiencies (iron, folate and B12), pro-inflammatory condition, and poor response to erythropoietin that results in erythropoiesis suppression.[16,20] Prevalence of anaemia has been found to be higher among CKD patients with T2DM compared to those without T2DM.[4,21] Among patients with T2DM, the prevalence of anaemia increases as their renal function deteriorates.[4-7,9,22] In the UK, a population-based study involving 234 patients with T2DM showed 5%, 16%, 23% and 46% of those with CKD stage 1, 2, 3 and 4/5 respectively had anaemia.[22]

Factors that were shown to be associated with anemia among patients with T2DM include older age, worsening renal function (i.e. lower eGFR, higher albuminuria and raised urine-albumin creatinine ratio), cardiovascular disease (stroke or ischaemic heart disease), peripheral vascular disease, lower weight or body mass index (BMI), lower diastolic blood pressure (DBP), longer duration of T2DM and not using angiotensin converting enzyme inhibitor.[2,4,7,9,23,24] However, there are inconsistent findings with regards to the association between gender and anemia among patients with T2DM and CKD. Anemia was significantly associated with females in studies done in Australia[2] and the UK[4] but a study in Hong Kong found its significant association with males[9].

To our knowledge, there is no published national study to determine the prevalence of anaemia among patients with T2DM and CKD in Malaysia. Thus, the burden of this problem remains unknown. Therefore, this study aimed to determine the prevalence of anaemia among patients with T2DM and CKD who received care

from the public primary care clinics in Peninsular Malaysia. Its associated factors were also examined as the findings could help the healthcare providers in identifying patients at higher risk for anaemia.

METHODS

This was a cross sectional observational study conducted at 20 government primary care clinics in Peninsular Malaysia which have resident Family Medicine Specialists. Fifteen clinics were selected using simple random sampling but five clinics were purposely chosen because they were managed by the researchers of this study. On average, there were 1,000-3,000 patients with T2DM registered at these clinics who stayed within 30 km radius from the respective clinics.

Study participants

Through systematic sampling, every third patient who attended for a follow-up visit during the data collection period (October to December 2015) were screened for their eligibility to be included in this study. The inclusion criteria were patients with T2DM aged 18 years old or more who had established CKD stage 1 to 5 and were able to understand either Malay or English language. Patients who were pregnant or had a recent delivery within previous three months or those with psychiatric illness or known anaemia secondary to any blood disorder such as thalassemia were excluded from this study. Eligible patients were invited to participate in the study by research assistants in the respective clinics. A maximum of 8 patients recruited daily from each clinic and the recruitment process stopped once 50 patients were sampled. The minimum targeted sample size for this study was 1,000 with a design effect of 2.0 which was sufficed to give 4% precision from the expected prevalence of anaemia of 30%. All patients who agreed to participate were interviewed for their socio-

demographic profiles and medical histories by the respective research assistants. Subsequently, their clinical and biomedical profiles were collected from their medical records. Blood taking for renal profiles or full blood count (FBC) was done if there was no recent result within the past six months.

Patient and Public Involvement

Patients with T2DM and CKD from each clinics were not involved in the design of the study and the recruitment of the participants. The results of the blood test were immediately informed to the study participants and appropriate management based on the results was carried out by the attending physician. Since this was a cross-sectional study, their involvement only during their clinic visit when they completed the self-administered questionnaire.

Definition of variables

Anaemia was defined based on KDIGO clinical practice guideline for anaemia in CKD when Hb <13.0 g/dL in males and <12.0 g/dL in females (KDIGO, 2012). Using estimated glomerular filtration rate (eGFR) and evidence of kidney damage (e.g. microalbuminuria or proteinuria), CKD was classified into: Stage 1: eGFR ≥90 ml/min/1.73m^2 with albuminuria or proteinuria, Stage 2: eGFR 60-89 ml/min/1.73m^2 with albuminuria or proteinuria, Stage 3a: eGFR 45-59 ml/min/1.73m^2, Stage 3b: eGFR 30-44 ml/min/1.73m^2, Stage 4: eGFR 15-29 ml/min/1.73m^2, and Stage 5: eGFR <15 ml/min/1.73m^2. Presence of co-morbidities and complications were determined through history taking or documentation in the medical records.

Statistical analysis

The statistical analysis was carried out using IBM SPSS Statistics version 22. All statistical analyses were performed at the 5% significance level. Findings were presented descriptively using mean (standard deviation, SD) and median (inter

quartile range, IQR) for normally and non-normally distributed continuous variables respectively, while results of categorical variables were presented as frequencies (n) and percentages (%). Multiple logistic regression (MLR) analysis with forward stepwise method was used to identify the associated factors of anaemia among patients with T2DM and CKD. Interactions between predictor variables and model fitness were also assessed.

Ethical consideration

This study obtained its approval from the Malaysian Medical Research and Ethics Committee (MREC) (NMRR-15-660-24324) and only patients who gave their written consent were included into this study. Anaemic patients found through this study were treated according to the recommended practice and referred to the nephrologist when indicated.

RESULTS

The mean (SD) age of the participants was 60.5 (9.5) years old (Table 1). The majority of the participants were Malays (75.2%). Males (56.2%) were slightly more than females (53.8%). The median (IQR) for duration of diabetes and CKD were 7.0 (8.0) years and 2.0 (3.0) years respectively, with median (IQR) of eGFR was 55.0 (34.0) ml/min/1.73m^2. The most common comorbidities were hypertension (88.5%) and dyslipidaemia (82.7%) (Table 2).

The prevalence of anemia among T2DM patients with CKD was 31.7% (256/808) with most of the anaemic patients had mild anaemia (61.5%, 166/256) and morphological classification of normocytic normochromic anaemia (58.7%, 148/252). The mean (SD) Hb of all patients was 13.18 (1.81) g/dL.

The prevalence of anaemia among T2DM patients with CKD 1, 2, 3a, 3b, 4 and 5 were 12.7%, 19.1%, 29.6%, 46.4%, 68.3% and 70.0% respectively (Table 3). Table 3 also shows that more patients had Hb <10 g/dL as stages of CKD increased, particularly those with CKD stage 4 and 5. Normocytic hypochromic anaemia also appeared to be more common at advanced stages of CKD, whereas microcytic hypochromic anemia was more common at earlier stages of CKD (Table 3).

Majority of the anaemic patients (88.7%, 227/256) had unrecognized anaemia as they were not known to have anaemia prior to this study (Table 1). Among 36 patients who had documented history of anaemia prior to the study, 80.6% (29/36) were still anaemic. Only about a half (15/29) of these patients received iron therapy.

Table 4 showed the results of the multivariate regression analysis. Female (AOR: 1.56, 95% CI: 1.12-2.21, p=0.009) and those with older age (AOR: 1.04, 95% CI: 1.01-1.06, p<0.001), CKD Stage 3a (AOR: 2.47; 95% CI: 1.25-4.87, p=0.009), CKD Stage 3b (AOR: 4.36; 95% CI: 2.14-8.85, p<0.001), CKD Stage 4 (AOR: 10.12; 95% CI: 4.36-23.47, p<0.001), CKD Stage 5 (AOR: 10.80; 95% CI: 3.32-35.11, p<0.001) and foot complication (AOR 3.12, 95% CI: 1.51-6.46, p=0.002) were more likely to have anaemia. Having higher body mass index (adjusted OR 0.95, 95% CI: 0.92-0.99, p=0.012) and higher diastolic blood pressure (adjusted OR 0.97, 95% CI: 0.95-0.99, p<0.001) were associated with lower odds to have anaemia.

DISCUSSION

Overall, patients with T2DM and CKD in this study were slightly different than general diabetes patients from the national studies in Malaysia.[12,13,15,25] Unlike our study which recruited patients from primary care clinics only, the previous Malaysian studies mostly include patients from the tertiary centres. Therefore the study

populations were different in terms of gender, ethnicity, duration of diabetes and severity of disease.[12,14,25] Males were slightly more than females in our study, whereas females were predominant in the other national studies. The Malays appear to be substantially more represented and this finding is consistent with Chew et al. (2011).[13] In this latter study, poor renal function was found to be more common among the Malays than the other ethnics.[13] Our participants also had longer duration of diabetes than the general diabetes patients. This is expected since nephropathy is more common as diabetes progresses and substantial proportion of patients with T2DM in Malaysia are on long term follow-up at primary care clinics.

In this study, the prevalence of anemia among patients with T2DM and CKD at primary care clinics was 31.7%. This prevalence was lower than another local study (39.4%) by Thambiah et al. (2015) which was done among 165 T2DM patients with CKD at the endocrine clinic of a tertiary hospital in Putrajaya, the federal administrative centre of Malaysia.[26] The difference in the prevalence may be because of the difference in the study sites. Higher prevalence of anaemia were also found in studies done at tertiary centres worldwide that ranged between 39.0% and 58.0%.[4,5,7,9] Nevertheless, the prevalence of anaemia in the current study was almost similar with a study done in 11 European countries (34.0%) involving 1205 patients with T2DM and CKD who were recruited from primary, secondary and tertiary settings.[8] This European study used the same definition of anaemia as our study.[8] Two other studies done among patients with diabetic nephropathy in the UK[6] and Japan[10] demonstrated even lower prevalence of anaemia compared to others in which both studies showed a prevalence of 26.0% .

Most of the patients with anaemia in this study had normocytic normochromic anaemia especially those at advanced stages of CKD. This morphological

classification of anaemia has been shown to be common not just among patients with CKD[27] in general but those with diabetic nephropathy[9,26] as well. This is due to impaired renal erythropoietin production and response to erythropoietin[28]. Nevertheless, microcytic hypochromic anaemia was quite prevalent particularly at earlier stages of CKD (CKD stage 1-3). Since thalassemia can cause this type of anaemia and is common in Southeast Asia like Malaysia, it is important to differentiate between patients with this illness and iron deficiency who require iron therapy.[29] Furthermore, patients with diabetes and CKD may have functional iron deficiency which requires replenishment of iron store before they could have definitive treatment of persistent renal anaemia with erythropoiesis-stimulating agents (ESA).[19,28] The iron therapy can be initiated even at primary care setting and since most anaemic patients had mild anaemia, as shown by the current study and Thambiah et al. (2015)[26], treatment with oral therapy may be suffice.

The current study also highlights a high prevalence of unrecognised anaemia among patients with T2DM and CKD whereby 88.7% of the anaemic patients were not diagnosed to have anaemia prior to this study. This prevalence of unrecognised anaemia is substantially higher than those in the western countries, which was less than 25%.[22,30] The finding of this current study suggests inadequate screening to detect anemia among patients with T2DM and CKD in Malaysian government primary care clinics. At present, screening for anaemia is not regularly practiced and, there is a need to review our management. In addition, 80.6% of the participants with previous history of anaemia were still anaemic at the time of the study whereby 51.7% of them received iron therapy but unable to restore normal level of Hb. About 48.3% of them were untreated. These findings indicate inadequate treatment of anaemia as similarly shown by Steven et al. (2010) which found only 22% of their

known anaemic patients treated with erythropoiesis-stimulating agents (ESA) or iron therapy.[8] In this current study, more patients with CKD stage 4 and 5 had Hb of less 10 g/dL, which is when ESA treatment is indicated. Nevertheless, iron therapy should be initiated at primary care clinic to replenish their iron stores before referring them to nephrologists for ESA treatment.[1,19,28] This undetected, uninvestigated and undertreated anaemia is worrisome since it may worsen their renal function and causes adverse cardiovascular outcomes.[28] Having a system to ensure screening of anaemia among at-risk patients will not produce favourable outcomes if anaemia is still not being properly managed. Since undertreated anaemia could be caused by many factors which include inadequate access to laboratory facilities, inadequate knowledge among doctors, patients' non-compliance, and cost of investigations and treatment, managing anaemia in patients with T2DM and CKD at primary health clinics is indeed challenging. Thus, further studies are required to confirm the actual causes of inadequate treatment of anaemia at primary care so that areas for improvement can be identified.

The mean Hb level of patients with T2DM and CKD in this study was almost similar with patients with diabetic nephropathy in Japan and European countries which was around 13 g/dL.[8,10] Hence, screening for all diabetic patients with CKD may not be cost effective. Nevertheless, this study highlights the associated factors that may alert the treating doctors of those who require screening for anaemia. These factors include increasing age, female gender, CKD stage 3 and above, presence of foot complication, lower BMI and lower diastolic blood pressure. Similarly, previous studies have shown significant associations of anaemia with older age[9,24], female gender[4], lower BMI and diastolic blood pressure[7] as well as higher stage of CKD[4,9]. A study done at a nephrology clinic in Greece by Loutradis et al.

(2016) also showed that anaemia was more prevalent among diabetic patients with CKD stage 3 compared to the non-diabetic with equivalent CKD stage.[31] These findings were in line with the recommendations by KDIGO (2012) in which asymptomatic patients with CKD stage 3 should be screened for anaemia yearly and more often for those with CKD stage 4 and 5.[19] In view of this, anaemia screening through simple FBC, which is available at every government primary care clinics in Malaysia, can be incorporated into the recommended yearly assessment of patients with diabetes but targeting to those with the significant factors found in this study. This approach could improve detection of patients who need further investigations and treatment without significant increase in the health care cost as the cost of FBC testing is low. Furthermore, the increased cost of an effective screening would be balanced by the reduction in the cost of treatment for the associated complications. Nevertheless, further study is still required to assess the cost-effectiveness of such practice.

This was the first nationwide study in Malaysia to examine the prevalence of anaemia among patients with T2DM and CKD involving a large sample from multi-centres in the public primary care setting. This study was able to identify those who require the simple FBC screening to improve the care of such patients. However, there are several limitations exist. Firstly, this study did not include diabetes patients from the private primary care clinics and those in the east of Malaysia. Therefore, the representativeness and generalisability of the findings ~~are~~ may be limited. The prevalence of anaemia among patients with T2DM and CKD at primary care settings in the whole Malaysia may also be underestimated. It is suggested that similar study could be extended by including patients from the private primary care clinics and those from the east Malaysia to verify our findings. Secondly, this study was not able

to identify the aetiology of the anaemia. The possible causes of anaemia were postulated just based on the morphology classification of anaemia, however it is inadequate as causes of anaemia are many and do not necessarily due to iron deficiency or CKD only. Lastly the cross-sectional design of the study could not determine the direction of relationship between anaemia and the associated factors. Nonetheless, our findings could provide a groundwork for future studies on anaemia among patients with T2DM and CKD in general.

CONCLUSION

Prevalence of anaemia among patients with T2DM and CKD in primary care setting was common and majority was unrecognized. Most of the anaemic patients had mild, normocytic hypochromic anaemia, but substantial proportions of those at advanced stage of CKD had moderate to severe anaemia and microcytic hypochromic anaemia. Inadequate treatment of anaemia was also prevalent. Therefore, screening of anaemia among patients with T2DM and CKD should be incorporated into the routine yearly assessment particularly for older age patients, females as well as those with CKD stage 3 and above, foot complication, lower BMI and lower DBP. It is hopeful, this will lead to early treatment and hence improve the overall care of patients with T2DM and CKD.

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DATA SHARING STATEMENT

Data collected from this study can be obtained from the corresponding author upon the agreement from the research team.

AUTHORS' CONTRIBUTION

All authors contributed in the conception and design of the study. II, AMA, NA, NS and JHA involved in the data acquisition. HT, NAM and MRAR analysed and interpreted the data. All authors involved in the appraisal of the findings, the drafting of the article and the final approval of the submitted version. All authors agreed to be accountable for this work.

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Table 1: Bivariate analysis of the participants' demographic and clinical characteristics with anaemia status

Characteristics	Overall n (%) or mean (SD) or median (IQR)	With anaemia n (%) or mean (SD) or median (IQR)	Without anaemia n (%) or mean (SD) or median (IQR)	p-value*
Age (years) (n=808)				
Mean (SD)	60.5 (9.5)	63.9 (9.1)	58.9 (9.2)	**<0.001**[a]
<60	378 (46.8)	87 (23.0)	291 (77.0)	
≥60	430 (53.2)	169 (39.3)	261 (60.7)	
Gender (n=808)				
Male	454 (56.2)	129 (28.4)	325 (71.6)	**0.024**[b]
Female	354 (43.8)	127 (35.9)	227 (64.1)	
Race (n=808)				
Malay	608 (75.2)	193 (31.7)	415 (68.3)	0.949[b]
Non-Malay	200 (24.8)	63 (31.5)	137 (68.5)	
Body mass index (kg/m²) (n=805)				
Mean (SD)	28.4 (4.8)	27.4 (5.0)	28.8 (4.7)	**<0.001**[a]
Underweight or normal (≤22.9)	94 (11.7)	53 (56.4)	41 (43.6)	
Overweight (23.0-27.4)	277 (34.4)	80 (28.9)	197 (71.1)	
Obese (≥27.5)	434 (53.9)	121 (27.9)	313 (72.1)	
Duration of T2DM (years) (n=807)				
Median (IQR)	7.0 (8.0)	9.0 (9.0)	7.0 (8.0)	**<0.001**[c]
< 5	231 (28.6)	49 (21.2)	182 (78.8)	
5 - 10	310 (38.4)	106 (34.2)	204 (65.8)	
> 10	266 (33.0)	101 (38.0)	165 (62.0)	
HbA1c (%) (n=729)				
Median (IQR)	8.2 (3.2)	8.2 (2.9)	8.2 (3.4)	0.301[c]
≤6.5	122 (16.7)	33 (27.0)	89 (73.0)	
6.6 – 7.0	85 (11.7)	36 (42.4)	49 (57.6)	
7.1 – 8.0	136 (18.7)	39 (28.7)	97 (71.3)	
≥8.1	386 (52.9)	117 (30.3)	269 (69.7)	
Fasting blood sugar (mmol/L) (n=538)				
Median (IQR)	7.9 (4.5)	7.4 (4.4)	8.0 (4.4)	**0.007**[c]
≤4.3	23 (4.3)	16 (69.6)	7 (30.4)	
4.4 – 7.0	184 (34.2)	57 (31.0)	127 (69.0)	
≥7.1	331 (61.5)	94 (28.4)	237 (71.6)	
Systolic blood pressure (mmHg) (n=807)				
Median (IQR)	140.0 (20.0)	140.0 (23.0)	140.0 (20.0)	0.433[c]
≤135	338 (41.9)	102 (30.2)	236 (69.8)	
≥136	469 (58.1)	154 (32.8)	315 (67.2)	
Diastolic blood pressure (mmHg) (n=807)				
Median (IQR)	80.0 (16.0)	77.0 (10.0)	80.0 (17.0)	**<0.001**[c]
≤75	298 (36.9)	124 (41.6)	174 (58.4)	
≥76	509 (63.1)	132 (25.9)	377 (74.1)	

Known anaemia (n=808)				
No	772 (95.5)	227 (29.4)	545 (70.6)	**<0.001**[b]
Yes	36 (4.5)	29 (80.6)	7 (19.4)	
Duration of CKD (years) (n=801)				
Median (IQR)	2.0 (3.0)	2.5 (3.3)	2.0 (2.0)	**<0.001**[c]

*Significance <0.05
[a]Independent t-test
[b]Chi-square test
[c]Mann-Whitney test

Table 2: Bivariate analysis of the participants' comorbidities and complications with anaemia status

Comorbidities and Complications*	All (N=808)		With anaemia (n=256)		Without anaemia (n=552)		p-value[d]
	N	%	n	%	n	%	
Hypertension	715	88.5	232	90.6	483	87.5	0.195[e]
Dyslipidaemia	668	82.7	205	80.1	463	83.9	0.184[e]
Ischaemic heart disease	83	10.3	37	14.3	46	8.3	**0.008**[e]
Heart failure	22	2.7	13	5.1	9	1.6	**0.005**[e]
Myocardial infarction	11	1.4	5	2.0	6	1.1	0.508[f]
Angina	9	1.1	4	1.6	5	0.9	0.640[f]
Cerebrovascular accident	36	4.5	18	7.0	18	3.3	**0.016**[e]
Peripheral vascular disease	5	0.6	1	0.4	4	0.7	0.935[f]
Chronic kidney disease							
Stage 1	102	12.6	13	5.1	89	16.1	**<0.001**[e]
Stage 2	230	28.5	44	17.2	186	33.7	
Stage 3a	240	29.7	71	27.7	169	30.6	
Stage 3b	153	18.9	71	27.7	82	14.9	
Stage 4	63	7.8	43	16.8	20	3.6	
Stage 5	20	2.5	14	5.5	6	1.1	
Retinopathy	151	18.7	64	25.0	87	15.8	**0.002**[e]
Neuropathy	119	14.7	41	16.0	78	14.1	0.482[e]
Erectile dysfunction (n=454)	54	11.9	18	14.0	36	11.1	0.393[e]
Foot complication	41	5.1	26	10.2	15	2.7	**<0.001**[e]
Others*[$]	192	23.8	71	27.7	121	21.9	0.071[e]
Cataract	18	2.2	7	2.7	11	2.0	0.506[e]
Osteoarthritis	17	2.1	5	2.0	12	2.2	0.839[e]
Spine problems[a]	10	1.2	3	1.2	7	1.3	1.000[f]
Gout arthritis	46	5.7	20	7.8	26	4.7	0.077[e]
Malignancy	14	1.7	4	1.6	10	1.8	1.000[f]
Asthma	20	2.5	4	1.6	16	2.9	0.255[e]
Renal calculi	17	2.1	3	1.2	14	2.5	0.209[e]
Upper gastrointestinal tract problems[b]	19	2.4	9	3.5	10	1.8	0.137[e]
Thyroid problem[c]	12	1.5	2	0.8	10	1.8	0.416[f]

*Patients may have more than one comorbidity and complication
[$]For comorbidities and complications entered in free text
[a]Spondylosis, degenerative spine disease or prolapsed intervertebral disc
[b]Dyspepsia, gastritis, gastro-oesophageal reflux or peptic ulcer disease

[c]Hyperthroidism or hypothryroidism
[d]Significance <0.05
[e]Chi-square test
[f]Chi-square test with continuity correction

Table 3: Median haemoglobin, prevalence of anaemia and description of anaemia according to stages of chronic kidney disease (CKD)

	Stage 1 (n=102)	Stage 2 (n=230)	Stage 3a (n=240)	Stage 3b (n=153)	Stage 4 (n=63)	Stage 5 (n=20)
Haemoglobin (g/dL) [Median (IQR)]	14.0 (2.2)	13.6 (1.8)	13.4 (2.2)	12.7 (2.3)	11.2 (2.6)	11.8 (3.5)
Anaemia (NFK-KDOQI) [n (%)]*	13 (12.7)	44 (19.1)	71 (29.6)	71 (46.4)	43 (70.0)	14 (70.0)
Severity of anaemia (n=256) [n (%)]						
Mild[a]	8 (61.5)	34 (77.3)	51 (71.8)	48 (67.6)	19 (44.2)	6 (42.9)
Moderate[b]	5 (38.5)	10 (22.7)	19 (26.8)	23 (32.4)	23 (53.5)	6 (42.9)
Severe[c]	0 (0)	0 (0)	1 (1.4)	0 (0)	1 (2.3)	2 (14.3)
Classification of anaemia based on morphology (n=252) [n (%)]						
Normocytic normochromic[d]	4 (30.8)	23 (52.3)	39 (54.9)	45 (63.4)	29 (70.7)	8 (66.7)
Microcytic hypochromic[e]	6 (46.1)	10 (22.7)	18 (25.4)	15 (21.1)	5 (12.2)	1 (8.3)
Macrocytic[f]	1 (7.7)	3 (6.8)	5 (7.0)	5 (7.0)	3 (7.3)	2 (16.7)
Others	2 (15.4)	8 (18.2)	9 (12.7)	6 (8.5)	4 (9.8)	1 (8.3)
Anaemia (Hb<10 g/dL) [n (%)]	1 (1.0)	1 (0.4)	6 (2.5)	8 (5.2)	13 (20.6)	6 (30.0)

*Hb <12 g/dL for females and <13 g/dL for males

[a]Mild: Hb 11.0-12.9 g/dL for males and Hb 11.0-11.9 g/dL for females; [b]Moderate: Hb 8.0-10.9 g/dL; [c]Severe: Hb ≤7.9 g/dL

[d]Normocytic normochromic: MCV 80-95 fL and MCH ≥27 pg; [e]Microcytic hypochromic: MCV <80 fL and MCH <27 pg; [f]Macrocytic: MCV >95 fL

Table 4: Multiple logistic regression (MLR) of the participants' characteristics with anaemia status

Variables (n=804)	B	Wald	Adjusted odds ratio	95% CI		p-value
Age (years)	0.03	10.34	1.04	1.01	1.06	0.001
Body mass index (kg/m^2)	-0.05	6.37	0.95	0.92	0.99	0.012
Diastolic blood pressure (mmHg)	-0.03	12.67	0.97	0.95	0.99	<0.001
Female [Male]	0.45	6.77	1.57	1.12	2.21	0.009
Stages of chronic kidney disease						
• Stage 2 [Stage 1]	0.35	0.98	1.42	0.71	2.84	0.323
• Stage 3a [Stage 1]	0.90	6.78	2.47	1.25	4.87	0.009
• Stage 3b [Stage 1]	1.47	16.56	4.36	2.14	8.85	<0.001
• Stage 4 [Stage 1]	2.31	29.07	10.12	4.36	23.47	<0.001
• Stage 5 [Stage 1]	2.38	15.65	10.80	3.32	35.11	<0.001
Presence of foot complication [No]	1.14	9.44	3.12	1.51	6.46	0.002

MLR: Stepwise forward (likelihood ratio); 18 independent variables with p<0.25 in simple logistic regression were initially entered: age, gender, BMI, HbA1c, diastolic blood pressure, duration of T2DM, duration of CKD, CKD stages, and presence of heart failure, ischaemic heart disease, cerebrovascular accident, hypertension, dyslipidaemia, retinopathy, foot complications, gouty arthritis, renal calculi and upper GIT problems. Constant: -0.289; No two-way interactions; Variables were not correlated; Model was fit: Hosmer and Lameshow test, p=0.539; Overall correct percentage: 75.1%; Area under ROC curve: 0.759 (95% CI: 0.724, 0.795; p<0.001)

STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology*

Checklist for cohort, case-control, and cross-sectional studies (combined)

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2-3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5-6
Objectives	3	State specific objectives, including any pre-specified hypotheses	6
Methods			**7**
Study design	4	Present key elements of study design early in the paper	
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	7
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7-8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*7-8*
Bias	9	Describe any efforts to address potential sources of bias	7
Study size	10	Explain how the study size was arrived at	7
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	
		(*b*) Describe any methods used to examine subgroups and interactions	
		(*c*) Explain how missing data were addressed	
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed	8

		Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	9
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	9
		(b) Indicate number of participants with missing data for each variable of interest	
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	9
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
Discussion			
Key results	18	Summarise key results with reference to study objectives	10
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	13-14
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	13-14
Generalisability	21	Discuss the generalisability (external validity) of the study results	13-14
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	4

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.
Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Anaemia among primary care patients with Type 2 Diabetes Mellitus (T2DM) and chronic kidney disease (CKD): a multi-centred cross-sectional study

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Anaemia among primary care patients with Type 2 Diabetes Mellitus (T2DM) and chronic kidney disease (CKD): a multi-centred cross-sectional study

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ABSTRACT

Objectives:

This study aimed to determine the prevalence of anaemia among patients with T2DM and CKD at primary care settings and its associated factors.

Design, setting and participants:

This cross-sectional study involved 808 adult patients with T2DM and CKD who were recruited via systematic sampling from 20 public primary care clinics in Peninsular Malaysia. Their socio-demographic, clinical and biomedical profiles were collected through interviews, examination of medical records and blood testing.

Results:

The prevalence of anemia was 31.7% (256/808). The anaemia was mainly mild (61.5%) and normocytic normochromic (58.7%). About 88.7% of the anaemic patients were not known to have anaemia prior to the study. Among 36 patients with documented history of anaemia, 80.6% were still anaemic and only a half received iron therapy. Multivariate regression analysis showed that female (AOR: 1.56, 95% CI: 1.12-2.21, p=0.009) and those with older age (AOR: 1.04, 95% CI: 1.01-1.06, p<0.001), CKD Stage 3a (AOR: 2.47; 95% CI: 1.25-4.87, p=0.009), CKD Stage 3b (AOR: 4.36; 95% CI: 2.14-8.85, p<0.001), CKD Stage 4 (AOR: 10.12; 95% CI: 4.36-23.47, p<0.001), CKD Stage 5 (AOR: 10.80; 95% CI: 3.32-35.11, p<0.001) and foot complication (AOR 3.12, 95% CI: 1.51-6.46, p=0.002) were more likely to have anaemia. Having higher body mass index

(adjusted OR 0.95, 95% CI: 0.92-0.99, p=0.012) and higher diastolic blood pressure (adjusted OR 0.97, 95% CI: 0.95-0.99, p<0.001) were associated with lower odds to have anaemia.

Conclusion:

Anaemia among patients with T2DM and CKD in primary care was common and the majority was unrecognised. Inadequate treatment of anaemia was also prevalent. Therefore, screening of anaemia should be incorporated into the routine assessment of diabetic complications particularly for those with significant associated factors. It is hoped that such strategy could lead to early treatment and hence improve their overall care.

Keywords: Anaemia, diabetes mellitus, chronic kidney disease, primary care

Trial registration number: NMRR-15-660-24324

Strength and limitations of this study:

1. This was the first nationwide study to examine the prevalence of anaemia among patients with T2DM and CKD involving a large sample from multi-centres in Malaysian primary care setting.
2. The findings help to identify those who require simple anaemia screening using full blood count and this potentially cost-saving approach of screening may improve the care of such patients.
3. The findings could also provide a groundwork for future studies as previous studies on anaemia among patients with T2DM and CKD are still limited.

4. Unfortunately in this study, the actual aetiology of the anaemia was not determined thus the anaemia may be caused by causes other than renal anaemia.
5. Since this study did not include patients from the private primary care clinics and those in the east of Malaysia (Sabah and Sarawak), the prevalence of anaemia may be underestimated.

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Conflicts of interest: None to declare

INTRODUCTION

Chronic kidney disease (CKD) is common among patients with type 2 diabetes mellitus (T2DM). It is defined when there is an irreversible loss of kidney function for at least three months.[1] The estimated glomerular filtration rate (eGFR) and evidence of kidney damage have been used to diagnose CKD, which can be divided into five stages based on the National Kidney Foundation-Kidney Disease Outcomes Quality Initiative (NKF-KDOQI) staging.[1] The estimated prevalence of diabetic nephropathy worldwide ranges between 26% and 58%.[2-10] However, the prevalence appears lower in Malaysia as it ranges between 7.8% and 34.9%.[11-15] This wide range of prevalence may be due to difference in study settings and definition used for diabetic nephropathy. Nevertheless, data from the National Diabetes Registry Malaysia in 2012 involving 657,839 registered T2DM patients from government health facilities including hospitals and primary care clinics showed a prevalence of 7.8%.[14]

CKD has been associated with anaemia, cardiovascular disease, bone metabolism abnormalities, metabolic acidosis and malnourishments. Among these, renal

anaemia has been increasingly studied over the past few years due to high all-cause mortality and cardiovascular morbidity associated with renal anaemia, as well as availability of erythropoietin stimulating agents (ESA) for treating renal anaemia.[16,17] In addition, anaemia has also been found to be a significant independent factor for progression of nephropathy to end stage renal failure among patients with T2DM.[18] Overall, renal anaemia carries significant burden to the sufferers as well as the healthcare system that bears the cost of care for patients with T2DM and CKD.

According to the Kidney Disease: Improving Global Outcome (KDIGO) Anemia Work Group, anaemia in CKD is when haemoglobin (Hb) level <13 g/dL for men and <12 g/dL for women.[19] It is caused by multiple pathophysiological mechanisms, which include erythropoietin deficiency, nutritional deficiencies (iron, folate and B12), pro-inflammatory condition, and poor response to erythropoietin that results in erythropoiesis suppression.[16,20] Prevalence of anaemia has been found to be higher among CKD patients with T2DM compared to those without T2DM.[4,21] Among patients with T2DM, the prevalence of anaemia increases as their renal function deteriorates.[4-7,9,22] In the UK, a population-based study involving 234 patients with T2DM showed 5%, 16%, 23% and 46% of those with CKD stage 1, 2, 3 and 4/5 respectively had anaemia.[22]

Factors that were shown to be associated with anemia among patients with T2DM include older age, worsening renal function (i.e. lower eGFR, higher albuminuria and raised urine-albumin creatinine ratio), cardiovascular disease (stroke or ischaemic heart disease), peripheral vascular disease, lower weight or body mass index (BMI), lower diastolic blood pressure (DBP), longer duration of T2DM and not using angiotensin converting enzyme inhibitor.[2,4,7,9,23,24] However, there are inconsistent findings with

regards to the association between gender and anemia among patients with T2DM and CKD. Anemia was significantly associated with females in studies done in Australia[2] and the UK[4] but a study in Hong Kong found its significant association with males[9].

Currently, screening for anaemia among these patients at the public primary care settings in Malaysia is not the standard of practice. Full blood count (FBC) would only be performed if clinically indicated. Due to financial limitation, asymptomatic patients and those without overt bleeding problems and constitutional or alarming symptoms would be normally prescribed with iron supplements. If there is no improvement with this therapy, they will then be subjected to further investigations to ascertain the aetiology of the anaemia. To our knowledge, there is no published national study to determine the prevalence of anaemia among patients with T2DM and CKD in Malaysia. Thus, the burden of this problem remains unknown. Therefore, this study aimed to determine the prevalence of anaemia among patients with T2DM and CKD who received care from the public primary care clinics in Peninsular Malaysia. Its associated factors were also examined as the findings could help the healthcare providers in identifying patients at higher risk for anaemia, thus screening of anaemia could be targeted to these patients.

METHODS

This was a cross sectional observational study conducted at 20 government primary care clinics in Peninsular Malaysia which have resident Family Medicine Specialists. Fifteen clinics were selected using simple random sampling but five clinics were purposely chosen because they were managed by the researchers of this study. On average, there

were 1,000-3,000 patients with T2DM registered at these clinics who stayed within 30 km radius from the respective clinics.

Study participants

Through systematic sampling, every third patient who attended for a follow-up visit during the data collection period (October to December 2015) was screened for their eligibility to be included in this study. The inclusion criteria were patients with T2DM aged 18 years old or more who had established CKD stage 1 to 5 and were able to understand either Malay or English language. Patients who were pregnant or had a recent delivery within previous three months or those with psychiatric illness or known anaemia secondary to any blood disorder such as thalassemia were excluded from this study. Eligible patients were invited to participate in the study by research assistants in the respective clinics. A maximum of 8 patients recruited daily from each clinic and the recruitment process stopped once 50 patients were sampled. The minimum targeted sample size for this study was 1,000 with a design effect of 2.0 which was sufficed to give 4% precision from the expected prevalence of anaemia of 30%. All patients who agreed to participate were interviewed for their socio-demographic profiles and medical histories by the respective research assistants. Subsequently, their clinical and biomedical profiles were collected from their medical records. Blood taking for renal profiles or full blood count (FBC) was done if there was no recent result within the past six months.

Patient and Public Involvement

Patients with T2DM and CKD from each clinics were not involved in the design of the study and the recruitment of the participants. The results of the blood test were immediately informed to the study participants and appropriate management based on

the results was carried out by the attending physician. Since this was a cross-sectional study, their involvement was only during their clinic visit when they completed the self-administered questionnaire.

Definition of variables

Anaemia was defined based on KDIGO clinical practice guideline for anaemia in CKD when Hb <13.0 g/dL in males and <12.0 g/dL in females (KDIGO, 2012). Using estimated glomerular filtration rate (eGFR) and evidence of kidney damage (e.g. microalbuminuria or proteinuria), CKD was classified into: Stage 1: eGFR ≥90 ml/min/1.73m^2 with albuminuria or proteinuria, Stage 2: eGFR 60-89 ml/min/1.73m^2 with albuminuria or proteinuria, Stage 3a: eGFR 45-59 ml/min/1.73m^2, Stage 3b: eGFR 30-44 ml/min/1.73m^2, Stage 4: eGFR 15-29 ml/min/1.73m^2, and Stage 5: eGFR <15 ml/min/1.73m^2. Presence of co-morbidities and complications were determined through history taking or documentation in the medical records.

Statistical analysis

The statistical analysis was carried out using IBM SPSS Statistics version 22. All statistical analyses were performed at the 5% significance level. Findings were presented descriptively using mean (standard deviation, SD) and median (inter quartile range, IQR) for normally and non-normally distributed continuous variables respectively, while results of categorical variables were presented as frequencies (n) and percentages (%). Multiple logistic regression (MLR) analysis with forward stepwise method was used to identify the associated factors of anaemia among patients with T2DM and CKD. Interactions between predictor variables and model fitness were also assessed.

Ethical consideration

This study obtained its approval from the Malaysian Medical Research and Ethics Committee (MREC) (NMRR-15-660-24324) and only patients who gave their written consent were included into this study. Anaemic patients found through this study were treated according to the recommended practice and referred to the nephrologist when indicated.

RESULTS

The mean (SD) age of the participants was 60.5 (9.5) years old (Table 1). The majority of the participants were Malays (75.2%). Males (56.2%) were slightly more than females (43.8%). The median (IQR) for duration of diabetes and CKD were 7.0 (8.0) years and 2.0 (3.0) years respectively, with median (IQR) of eGFR was 55.0 (34.0) ml/min/1.73m^2. More than a half of the patients were on either angiotensin-converting enzyme inhibitors or angiotensin receptor blockade (72.2%). There was no significant difference in the presence of anaemia between those who received this treatment and those without. The most common comorbidities were hypertension (88.5%) and dyslipidaemia (82.7%) (Table 2).

The prevalence of anemia among T2DM patients with CKD was 31.7% (256/808) with most of the anaemic patients having mild anaemia (61.5%, 166/256) and morphological classification of normocytic normochromic anaemia (58.7%, 148/252). The mean (SD) Hb of all patients was 13.18 (1.81) g/dL.

The prevalence of anaemia among T2DM patients with CKD 1, 2, 3a, 3b, 4 and 5 were 12.7%, 19.1%, 29.6%, 46.4%, 68.3% and 70.0% respectively (Table 3). Table 3 also shows that more patients had Hb <10 g/dL as stages of CKD increased, particularly

those with CKD stage 4 and 5. Normocytic hypochromic anaemia also appeared to be more common at advanced stages of CKD, whereas microcytic hypochromic anemia was more common at earlier stages of CKD (Table 3).

Majority of the anaemic patients (88.7%, 227/256) had unrecognized anaemia as they were not known to have anaemia prior to this study (Table 1). Among 36 patients who had documented history of anaemia prior to the study, 80.6% (29/36) were still anaemic. Only about a half (15/29) of these patients received iron therapy.

Table 4 showed the results of the multivariate regression analysis. Female (AOR: 1.56, 95% CI: 1.12-2.21, p=0.009) and those with older age (AOR: 1.04, 95% CI: 1.01-1.06, p<0.001), CKD Stage 3a (AOR: 2.47; 95% CI: 1.25-4.87, p=0.009), CKD Stage 3b (AOR: 4.36; 95% CI: 2.14-8.85, p<0.001), CKD Stage 4 (AOR: 10.12; 95% CI: 4.36-23.47, p<0.001), CKD Stage 5 (AOR: 10.80; 95% CI: 3.32-35.11, p<0.001) and foot complication (AOR 3.12, 95% CI: 1.51-6.46, p=0.002) were more likely to have anaemia. Having higher body mass index (adjusted OR 0.95, 95% CI: 0.92-0.99, p=0.012) and higher diastolic blood pressure (adjusted OR 0.97, 95% CI: 0.95-0.99, p<0.001) were associated with lower odds to have anaemia.

DISCUSSION

Overall, patients with T2DM and CKD in this study were slightly different than general diabetes patients from the national studies in Malaysia.[12,13,15,25] Unlike our study which recruited patients from primary care clinics only, the previous Malaysian studies mostly include patients from the tertiary centres. Therefore the study populations were different in terms of gender, ethnicity, duration of diabetes and severity of disease.[12,14,25] Males

were slightly more than females in our study, whereas females were predominant in the other national studies. The Malays appear to be substantially more represented and this finding is consistent with Chew et al. (2011).[13] In this latter study, poor renal function was found to be more common among the Malays than the other ethnics.[13] Our participants also had longer duration of diabetes than the general diabetes patients. This is expected since nephropathy is more common as diabetes progresses and substantial proportion of patients with T2DM in Malaysia are on long term follow-up at primary care clinics.

In this study, the prevalence of anemia among patients with T2DM and CKD at primary care clinics was 31.7%. This prevalence was lower than another local study (39.4%) by Thambiah et al. (2015) which was done among 165 T2DM patients with CKD at the endocrine clinic of a tertiary hospital in Putrajaya, the federal administrative centre of Malaysia.[26] The difference in the prevalence may be because of the difference in the study sites. Higher prevalence of anaemia was also found in studies done at tertiary centres worldwide that ranged between 39.0% and 58.0%.[4,5,7,9] Nevertheless, the prevalence of anaemia in the current study was almost similar with a study done in 11 European countries (34.0%) involving 1205 patients with T2DM and CKD who were recruited from primary, secondary and tertiary settings.[8] This European study used the same definition of anaemia as our study.[8] Two other studies done among patients with diabetic nephropathy in the UK[6] and Japan[10] demonstrated even lower prevalence of anaemia compared to others in which both studies showed a prevalence of 26.0% .

Most of the patients with anaemia in this study had normocytic normochromic anaemia especially those at advanced stages of CKD. This morphological classification of anaemia has been shown to be common not just among patients with CKD[27] in general

but those with diabetic nephropathy[9,26] as well. This is due to impaired renal erythropoietin production and response to erythropoietin[28]. Nevertheless, microcytic hypochromic anaemia was quite prevalent particularly at earlier stages of CKD (CKD stage 1-3). Since thalassemia can cause this type of anaemia and is common in Southeast Asia like Malaysia, it is important to differentiate between patients with this illness and iron deficiency who require iron therapy.[29] Furthermore, patients with diabetes and CKD may have functional iron deficiency which requires replenishment of iron store before they could have definitive treatment of persistent renal anaemia with erythropoiesis-stimulating agents (ESA).[19,28] The iron therapy can be initiated even at primary care setting and since most anaemic patients had mild anaemia, as shown by the current study and Thambiah et al. (2015)[26], treatment with oral therapy may be suffice.

The current study also highlights a high prevalence of unrecognised anaemia among patients with T2DM and CKD whereby 88.7% of the anaemic patients were not diagnosed to have anaemia prior to this study. This prevalence of unrecognised anaemia is substantially higher than those in the western countries, which was less than 25%.[22,30] The finding of this current study suggests inadequate screening to detect anemia among patients with T2DM and CKD in Malaysian government primary care clinics. At present, screening for anaemia is not regularly practiced and, there is a need to review our management. In addition, 80.6% of the participants with previous history of anaemia were still anaemic at the time of the study whereby 51.7% of them received iron therapy but unable to restore normal level of Hb. About 48.3% of them were untreated. These findings indicate inadequate treatment of anaemia as similarly shown by Stevens et al. (2010) which found only 22% of their known anaemic patients treated with erythropoiesis-

stimulating agents (ESA) or iron therapy.[8] In this current study, more patients with CKD stage 4 and 5 had Hb of less 10 g/dL, which is when ESA treatment is indicated. Nevertheless, iron therapy should be initiated at primary care clinic to replenish their iron stores before referring them to nephrologists for ESA treatment.[1,19,28] This undetected, uninvestigated and undertreated anaemia is worrisome since it may worsen their renal function and causes adverse cardiovascular outcomes.[28] Having a system to ensure screening of anaemia among at-risk patients will not produce favourable outcomes if anaemia is still not being properly managed. Since undertreated anaemia could be caused by many factors which include inadequate access to laboratory facilities, inadequate knowledge among doctors, patients' non-compliance, and cost of investigations and treatment, managing anaemia in patients with T2DM and CKD at primary health clinics is indeed challenging. Thus, further studies are required to confirm the actual causes of inadequate treatment of anaemia at primary care so that areas for improvement can be identified.

The mean Hb level of patients with T2DM and CKD in this study was almost similar with patients with diabetic nephropathy in Japan and European countries which was around 13 g/dL.[8,10] Hence, screening for all diabetic patients with CKD may not be cost effective. Nevertheless, this study highlights the associated factors that may alert the treating doctors of those who require screening for anaemia. These factors include increasing age, female gender, CKD stage 3 and above, presence of foot complication, lower BMI and lower diastolic blood pressure. Similarly, previous studies have shown significant associations of anaemia with older age[9,24], female gender[4], lower BMI and diastolic blood pressure[7] as well as higher stage of CKD[4,9]. A study done at a nephrology

clinic in Greece by Loutradis et al. (2016) also showed that anaemia was more prevalent among diabetic patients with CKD stage 3 compared to the non-diabetic with equivalent CKD stage.[31] These findings were in line with the recommendations by KDIGO (2012) in which asymptomatic patients with CKD stage 3 should be screened for anaemia yearly and more often for those with CKD stage 4 and 5.[19] In view of this, anaemia screening through simple FBC, which is available at every government primary care clinics in Malaysia, can be incorporated into the recommended yearly assessment of patients with diabetes but targeting to those with the significant factors found in this study. This approach could improve detection of patients who need further investigations and treatment without significant increase in the health care cost as the cost of FBC testing is low. Furthermore, the increased cost of an effective screening would be balanced by the reduction in the cost of treatment for the associated complications. Nevertheless, further study is still required to assess the cost-effectiveness of such practice.

This was the first nationwide study in Malaysia to examine the prevalence of anaemia among patients with T2DM and CKD involving a large sample from multi-centres in the public primary care setting. This study was able to identify those who require the simple FBC screening to improve the care of such patients. However, there are several limitations. Firstly, this study did not include diabetes patients from the private primary care clinics and those in the east of Malaysia. Therefore, the representativeness and generalisability of the findings may be limited. The prevalence of anaemia among patients with T2DM and CKD at primary care settings in the whole Malaysia may also be underestimated. It is suggested that similar study could be extended by including patients from the private primary care clinics and those from the east Malaysia to verify our

findings. Secondly, this study was not able to identify the aetiology of the anaemia. The possible causes of anaemia were postulated just based on the morphology classification of anaemia, however it is inadequate as causes of anaemia are many and are not necessarily due to iron deficiency or CKD only. Lastly the cross-sectional design of the study could not determine the direction of relationship between anaemia and the associated factors. Nonetheless, our findings could provide a groundwork for future studies on anaemia among patients with T2DM and CKD in general.

CONCLUSION

Anaemia among patients with T2DM and CKD in primary care setting was common and majority was unrecognized. Most of the anaemic patients had mild, normocytic hypochromic anaemia, but substantial proportions of those at advanced stage of CKD had moderate to severe anaemia and microcytic hypochromic anaemia. Inadequate treatment of anaemia was also prevalent. Therefore, screening of anaemia among patients with T2DM and CKD should be incorporated into the routine yearly assessment particularly for older age patients, females as well as those with CKD stage 3 and above, foot complication, lower BMI and lower DBP. It is hopeful, this will lead to early treatment and hence improve the overall care of patients with T2DM and CKD.

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DATA SHARING STATEMENT

Data collected from this study can be obtained from the corresponding author upon the agreement from the research team.

AUTHORS' CONTRIBUTION

All authors contributed in the conception and design of the study. II, AMA, NA, NS and JHA involved in the data acquisition. HT, NAM and MRAR analysed and interpreted the data. All authors involved in the appraisal of the findings, the drafting of the article and the final approval of the submitted version. All authors agreed to be accountable for this work.

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Table 1: Bivariate analysis of the participants' demographic and clinical characteristics with anaemia status

Characteristics	Overall	With anaemia	Without anaemia	p-value*

	n (%) or mean (SD) or median (IQR)	n (%) or mean (SD) or median (IQR)	n (%) or mean (SD) or median (IQR)	
Age (years) (n=808)				
Mean (SD)	60.5 (9.5)	63.9 (9.1)	58.9 (9.2)	**<0.001[a]**
<60	378 (46.8)	87 (23.0)	291 (77.0)	
≥60	430 (53.2)	169 (39.3)	261 (60.7)	
Gender (n=808)				
Male	454 (56.2)	129 (28.4)	325 (71.6)	**0.024[b]**
Female	354 (43.8)	127 (35.9)	227 (64.1)	
Race (n=808)				
Malay	608 (75.2)	193 (31.7)	415 (68.3)	0.949[b]
Non-Malay	200 (24.8)	63 (31.5)	137 (68.5)	
Body mass index (kg/m²) (n=805)				
Mean (SD)	28.4 (4.8)	27.4 (5.0)	28.8 (4.7)	**<0.001[a]**
Underweight or normal (≤22.9)	94 (11.7)	53 (56.4)	41 (43.6)	
Overweight (23.0-27.4)	277 (34.4)	80 (28.9)	197 (71.1)	
Obese (≥27.5)	434 (53.9)	121 (27.9)	313 (72.1)	
Duration of T2DM (years) (n=807)				
Median (IQR)	7.0 (8.0)	9.0 (9.0)	7.0 (8.0)	**<0.001[c]**
< 5	231 (28.6)	49 (21.2)	182 (78.8)	
5 - 10	310 (38.4)	106 (34.2)	204 (65.8)	
> 10	266 (33.0)	101 (38.0)	165 (62.0)	
HbA1c (%) (n=729)				
Median (IQR)	8.2 (3.2)	8.2 (2.9)	8.2 (3.4)	0.301[c]
≤6.5	122 (16.7)	33 (27.0)	89 (73.0)	
6.6 – 7.0	85 (11.7)	36 (42.4)	49 (57.6)	
7.1 – 8.0	136 (18.7)	39 (28.7)	97 (71.3)	
≥8.1	386 (52.9)	117 (30.3)	269 (69.7)	
Fasting blood sugar (mmol/L) (n=538)				
Median (IQR)	7.9 (4.5)	7.4 (4.4)	8.0 (4.4)	**0.007[c]**
≤4.3	23 (4.3)	16 (69.6)	7 (30.4)	
4.4 – 7.0	184 (34.2)	57 (31.0)	127 (69.0)	
≥7.1	331 (61.5)	94 (28.4)	237 (71.6)	
Systolic blood pressure (mmHg) (n=807)				
Median (IQR)	140.0 (20.0)	140.0 (23.0)	140.0 (20.0)	0.433[c]
≤135	338 (41.9)	102 (30.2)	236 (69.8)	
≥136	469 (58.1)	154 (32.8)	315 (67.2)	
Diastolic blood pressure (mmHg) (n=807)				
Median (IQR)	80.0 (16.0)	77.0 (10.0)	80.0 (17.0)	**<0.001[c]**
≤75	298 (36.9)	124 (41.6)	174 (58.4)	
≥76	509 (63.1)	132 (25.9)	377 (74.1)	
Known anaemia (n=808)				
No	772 (95.5)	227 (29.4)	545 (70.6)	**<0.001[b]**
Yes	36 (4.5)	29 (80.6)	7 (19.4)	
Duration of CKD (years) (n=801)				
Median (IQR)	2.0 (3.0)	2.5 (3.3)	2.0 (2.0)	**<0.001[c]**
Treated with ACE-i or ARB				
No	225 (27.8)	71 (31.6)	154 (68.4)	0.961[c]
Yes	583 (72.2)	185 (31.7)	398 (68.3)	

*Significance <0.05

[a]Independent t-test; [b]Chi-square test; [c]Mann-Whitney test

SD: Standard deviation; IQR: Inter quartile range; T2DM: Type 2 diabetes mellitus; CKD: Chronic kidney disease; ACE-I: Angiotensin converting enzyme inhibitor; ARB: Angiotensin II receptor blocker

Table 2: Bivariate analysis of the participants' comorbidities and complications with anaemia status

Comorbidities and Complications*	All (N=808)		With anaemia (n=256)		Without anaemia (n=552)		p-value[d]
	N	%	n	%	n	%	
Hypertension	715	88.5	232	90.6	483	87.5	0.195[e]
Dyslipidaemia	668	82.7	205	80.1	463	83.9	0.184[e]
Ischaemic heart disease	83	10.3	37	14.3	46	8.3	**0.008[e]**
Heart failure	22	2.7	13	5.1	9	1.6	**0.005[e]**
Myocardial infarction	11	1.4	5	2.0	6	1.1	0.508[f]
Angina	9	1.1	4	1.6	5	0.9	0.640[f]
Cerebrovascular accident	36	4.5	18	7.0	18	3.3	**0.016[e]**
Peripheral vascular disease	5	0.6	1	0.4	4	0.7	0.935[f]
Chronic kidney disease							
Stage 1	102	12.6	13	5.1	89	16.1	**<0.001[e]**
Stage 2	230	28.5	44	17.2	186	33.7	
Stage 3a	240	29.7	71	27.7	169	30.6	
Stage 3b	153	18.9	71	27.7	82	14.9	
Stage 4	63	7.8	43	16.8	20	3.6	
Stage 5	20	2.5	14	5.5	6	1.1	
Retinopathy	151	18.7	64	25.0	87	15.8	**0.002[e]**
Neuropathy	119	14.7	41	16.0	78	14.1	0.482[e]
Erectile dysfunction (n=454)	54	11.9	18	14.0	36	11.1	0.393[e]
Foot complication	41	5.1	26	10.2	15	2.7	**<0.001[e]**
Others*[$]	192	23.8	71	27.7	121	21.9	0.071[e]
Cataract	18	2.2	7	2.7	11	2.0	0.506[e]
Osteoarthritis	17	2.1	5	2.0	12	2.2	0.839[e]
Spine problems[a]	10	1.2	3	1.2	7	1.3	1.000[f]
Gout arthritis	46	5.7	20	7.8	26	4.7	0.077[e]
Malignancy	14	1.7	4	1.6	10	1.8	1.000[f]
Asthma	20	2.5	4	1.6	16	2.9	0.255[e]
Renal calculi	17	2.1	3	1.2	14	2.5	0.209[e]
Upper gastrointestinal tract problems[b]	19	2.4	9	3.5	10	1.8	0.137[e]
Thyroid problem[c]	12	1.5	2	0.8	10	1.8	0.416[f]

*Patients may have more than one comorbidity and complication
[$]For comorbidities and complications entered in free text
[a]Spondylosis, degenerative spine disease or prolapsed intervertebral disc
[b]Dyspepsia, gastritis, gastro-oesophageal reflux or peptic ulcer disease
[c]Hyperthroidism or hypothryroidism
[d]Significance <0.05
[e]Chi-square test
[f]Chi-square test with continuity correction

Table 3: Median haemoglobin, prevalence of anaemia and description of anaemia according to stages of chronic kidney disease (CKD)

	Stage 1 (n=102)	Stage 2 (n=230)	Stage 3a (n=240)	Stage 3b (n=153)	Stage 4 (n=63)	Stage 5 (n=20)
Haemoglobin (g/dL) [Median (IQR)]	14.0 (2.2)	13.6 (1.8)	13.4 (2.2)	12.7 (2.3)	11.2 (2.6)	11.8 (3.5)
Anaemia (NFK-KDOQI) [n (%)]*	13 (12.7)	44 (19.1)	71 (29.6)	71 (46.4)	43 (70.0)	14 (70.0)
Severity of anaemia (n=256) [n (%)]						
Mild[a]	8 (61.5)	34 (77.3)	51 (71.8)	48 (67.6)	19 (44.2)	6 (42.9)
Moderate[b]	5 (38.5)	10 (22.7)	19 (26.8)	23 (32.4)	23 (53.5)	6 (42.9)
Severe[c]	0 (0)	0 (0)	1 (1.4)	0 (0)	1 (2.3)	2 (14.3)
Classification of anaemia based on morphology (n=252) [n (%)]						
Normocytic normochromic[d]	4 (30.8)	23 (52.3)	39 (54.9)	45 (63.4)	29 (70.7)	8 (66.7)
Microcytic hypochromic[e]	6 (46.1)	10 (22.7)	18 (25.4)	15 (21.1)	5 (12.2)	1 (8.3)
Macrocytic[f]	1 (7.7)	3 (6.8)	5 (7.0)	5 (7.0)	3 (7.3)	2 (16.7)
Others	2 (15.4)	8 (18.2)	9 (12.7)	6 (8.5)	4 (9.8)	1 (8.3)
Anaemia (Hb<10 g/dL) [n (%)]	1 (1.0)	1 (0.4)	6 (2.5)	8 (5.2)	13 (20.6)	6 (30.0)

*Hb <12 g/dL for females and <13 g/dL for males

[a]Mild: Hb 11.0-12.9 g/dL for males and Hb 11.0-11.9 g/dL for females; [b]Moderate: Hb 8.0-10.9 g/dL; [c]Severe: Hb ≤7.9 g/dL

[d]Normocytic normochromic: MCV 80-95 fL and MCH ≥27 pg; [e]Microcytic hypochromic: MCV <80 fL and MCH <27 pg; [f]Macrocytic: MCV >95 fL

Table 4: Multiple logistic regression (MLR) of the participants' characteristics with anaemia status

Variables (n=804)	B	Wald	Adjusted odds ratio	95% CI		p-value
Age (years)	0.03	10.34	1.04	1.01	1.06	0.001
Body mass index (kg/m^2)	-0.05	6.37	0.95	0.92	0.99	0.012
Diastolic blood pressure (mmHg)	-0.03	12.67	0.97	0.95	0.99	<0.001
Female [Male]	0.45	6.77	1.57	1.12	2.21	0.009
Stages of chronic kidney disease						
• Stage 2 [Stage 1]	0.35	0.98	1.42	0.71	2.84	0.323
• Stage 3a [Stage 1]	0.90	6.78	2.47	1.25	4.87	0.009
• Stage 3b [Stage 1]	1.47	16.56	4.36	2.14	8.85	<0.001
• Stage 4 [Stage 1]	2.31	29.07	10.12	4.36	23.47	<0.001
• Stage 5 [Stage 1]	2.38	15.65	10.80	3.32	35.11	<0.001
Presence of foot complication [No]	1.14	9.44	3.12	1.51	6.46	0.002

MLR: Stepwise forward (likelihood ratio); 18 independent variables with p<0.25 in simple logistic regression were initially entered: age, gender, BMI, HbA1c, diastolic blood pressure, duration of T2DM, duration of CKD, CKD stages, and presence of heart failure, ischaemic heart disease, cerebrovascular accident, hypertension, dyslipidaemia, retinopathy, foot complications, gouty arthritis, renal calculi and upper GIT problems.

Constant: -0.289; No two-way interactions; Variables were not correlated; Model was fit: Hosmer and Lameshow test, p=0.539; Overall correct percentage: 75.1%; Area under ROC curve: 0.759 (95% CI: 0.724, 0.795; p<0.001)

STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology*

Checklist for cohort, case-control, and cross-sectional studies (combined)

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2-3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5-6
Objectives	3	State specific objectives, including any pre-specified hypotheses	6
Methods			**7**
Study design	4	Present key elements of study design early in the paper	
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	7
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7-8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*7-8*
Bias	9	Describe any efforts to address potential sources of bias	7
Study size	10	Explain how the study size was arrived at	7
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	
		(*b*) Describe any methods used to examine subgroups and interactions	
		(*c*) Explain how missing data were addressed	
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed	8

		Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	9
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	9
		(b) Indicate number of participants with missing data for each variable of interest	
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	9
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
Discussion			
Key results	18	Summarise key results with reference to study objectives	10
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	13-14
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	13-14
Generalisability	21	Discuss the generalisability (external validity) of the study results	13-14
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	4

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.
Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.